molecules, is the molecule the monomer or is it the polymer?

DR. MARCHAND: I use this term for infectious dose, because we don't know if it is a monomer, dimer, thrimer, or a ten thousand for each of them. The only thing we know, it's a clump that is infectious, a clump of atoms, and we don't know exactly what it is.

DR. GRAMMAR: How did you determine that there were 600 molecules?

DR. MARCHAND: The real term would be infectious dose. Okay? We do the same dilution assays as you've seen. We start with an extract that we make ten-fold dilutions, and we inject that to animals or we implant it in animals. And according to -- this is the type of Karber type of study that all the previous presenters talked about before me. The dilutions are given to animals, and you look how many of them give the disease, 50 percent of the disease, 50 percent of them are infected.

DR. GRAMMAR: Right. But I'm trying to figure out how many centigrams of protein is 600

molecules?

DR. MARCHAND: We don't know. Once again, we don't know what is the basic infectious molecule here. In some cell assays, they're expected to be dimers or monomers, but in reality, in the living animal model, we don't know exactly what is this basic unit of infection.

DR. TELLING: So there's some uncertainty there, but we are certain that we're not looking at molecules. This is not molecules.

DR. MARCHAND: Yes, these are not molecules. It's a wrong term, I must agree with that.

CHAIRMAN EDMISTON: Dr. Schonberger.

DR. SCHONBERGER: I was wondering if you could just clarify how you know you started out with ten to the ninth.

DR. MARCHAND: Once again, it's the way these experiments are designed by dilution methods. We plucked, reverse plucked the projection by diluting let's say by a million the extract, and you still have, let's say, a few animals, you know that you have at least an infectious particle in there. So the more

you concentrate the extract, maybe Dr. Prusiner will be able to explain it, but it's the Karber method that had been always used since the 40s. It's by dilution processes with exposing different series of animals.

CHAIRMAN EDMISTON: Ms. Sanithraj.

DR. MANGAIYARKARASI: Yes. You were talking about sensitivity. Is there any way we can know the specificity of the test model?

DR. MARCHAND: Each prion goes with its animal model. For instance, you cannot transfer them easily from one to the other, or not for all the prion strains available. It's a pair, and each pair of prion, the 263K prion goes with the golden hamster. And the RML strain goes with the Tg4053, or whatsoever. These are pairs that can get each other, and they don't have the same behavior, the same susceptibility.

Now, for instance, if we would like to define what is a proper challenge, let's say that a suggestion could be eight logs of the basic minimal ID100 percent that makes all the animals sick, so if we use eight logs of 8,000 infectious dose for one

because this model is less sensitive, we can use the same eight log of 600 molecules with another one, we would be able to standardize the inoculum under that consideration.

Now if we do that, we could also make available the capability to control or to compare reduction assays with, let's say, the Sub-35 prion-like molecule that is a fungal prion, and help us to compare one animal to the other through an independent prion-like molecule, for instance. So if we can -- if we would be in a situation to compare these models based on the infectious dose, that it is minimal to have 100 percent of animals because once again, what is guaranteed is the disease. Health is not guaranteed absence of prion with this disease.

Dr. EDMISTON: Are there any further questions? Yes.

DR. GRAMMAR: What do you use -- what's the full strength inoculum? Is it like ground up brain or what is it?

DR. MARCHAND: Generally, because the whole brain homogenate is syrupy, and it's lipidy,

it's kind of molasses-looking stuff, it's a 10 percent dilution.

DR. GRAMMAR: But it is just whole brain.

DR. MARCHAND: Whole brain blended and diluted to start with the 10 percent, and this 10 percent homogenate is generally varying between ten to the seventh, ten to the eleventh log of infection, infectious dose.

DR. GRAMMAR: What's the molecular weight of range of the proteins that are in the inoculum?

DR. MARCHAND: It's a soup of hundreds of proteins, and lipids, and what's --

DR. GRAMMAR: Like maybe 5,000? Like do you cut it so that only protein goes in, instead of glucose and all that stuff?

DR. MARCHAND: In some experiments, they have, especially the French group they use purified protein concentrate to do a lot of experiments, because it helps you quantify the protein numbers, and look at molecular weight. But most of the hamster assays are done to mimic, in part, what happens in the hospital when you poke a brain with a device because

you're doing a surgery, you have a mixture of lipid, of blood, glucose and brain extracts, so they're using brain extracts.

DR. GRAMMAR: Thank you.

CHAIRMAN EDMISTON: Are there any further questions for Dr. Marchand? Yes, Dr. Gordon.

DR. GORDON: I guess one of the concerns that I'm having is that one of the major issues with this has always been the prolonged incubation period that we have with human disease. And then it seems to me, I don't do animal experiments, that it's almost arbitrarily been set at 365 days that we're going to cut it off for animals. And part of it, obviously, is they don't live long enough. But the concern that I have and I wanted to address is that they could have sub-clinical disease you can't see with a microscope that might not present for four or five years, and you wouldn't know about it. But people live so much longer that they would get clinical disease, which would make this animal model, none of these animal models particularly accurate in predicting whether or not we're getting to a low enough level to actually

provide human protection.

DR. MARCHAND: You're absolutely right. If you look at healthy animals that survive a certain period to let's say verify or validate the hypothesis of safety, you need hundreds and hundreds of animals because there is a definite probability of having some disease and incubation in some of that. Now if you look, your end point is a diseased animal, you have a hard point on a curve that you can corroborate with another point on the curve and see how these things are actually in reduction if you expose them to processes.

CHAIRMAN EDMISTON: Yes, Dr. Dr. Telling.

DR. TELLING: Just a comment, I think what these assays tell you is that you get a certain level of inactivation based on how many orders of magnitude, and that's all it's telling you, four logs reduction, six logs reduction, what have you.

DR. MARCHAND: From the infection control standpoint, the number of log of reduction may be in part irrelevant because nobody would care for a 12 or a 15 log reduction if your device is still infectious

and has a probability of transmitting the disease one on two or one on four. Now what from the infection control standpoint, what we would like to see is a risk of transmitting disease of one in a million. This would be below the surgical risk, below the anesthetic risk, and even the risk of having a car accident and dying on your way to the hospital. This would be acceptable, so the log reduction in terms of predicting the risk is not necessarily what the infection control wants to see, but in terms of managing and defining how you can get down this risk with a process is one way to go.

CHAIRMAN EDMISTON: Dr. Arduino.

DR. ARDUINO: Well, what I see from a lot of these animal studies is we really don't have an end point, because we're arbitrarily cutting off at either one year for mice, or two years for hamsters. And yes, we see a disease in a number after X many days, and then there's nothing. But we know from dilutions that the more you dilute the product or the agent out, the longer the incubation period. So how -- well, you've got to either extend studies out or --

DR. MARCHAND: There's no predictive tailing effect when you're under the certainty of the minimum dose that tells you that your animal will be sick, which is the minimal infectious dose for 100 percent. Under that, you have no certainty of nothing.

CHAIRMAN EDMISTON: Let me ask a very quick question. Was your comment that the acceptable risk is somewhere less than one per million? Is that --

DR. MARCHAND: We do that actually with the SAL and sterilization. We accept a risk, the safety -- the sterilization level is a way to say that we accept a risk of one in a million.

CHAIRMAN EDMISTON: Do you accept the FDA's risk assessment data as it was presented this morning?

DR. MARCHAND: Yes, but I would have some comments to it. Now there is no human activity without some kind of risk. Just by walking here, you have the risk of dying of anything, you know, so what is an acceptable risk? Differs from one society to

the other.

Now if we cannot predict the risk of transmitting a disease, at least we can say that we accept a risk of transmission that is below, let's say one in a million, or 100,000, depends on the societies, but it's a way to look at -- when you take your car, you accept the risk of dying in a car accident.

CHAIRMAN EDMISTON: But relative to this discussion, TSE, in essence, we're at that risk now. That's the risk level we're at today. So, in essence, we're there.

DR. MARCHAND: Yes, we're around there.

CHAIRMAN EDMISTON: So you're not proposing we lower the risk any further. Are there any further questions for Dr. Marchand?

DR. MANGAIYARKARASI: The instance of somebody wanting one million in the population, and if we can reduce it further, than we can accept.

CHAIRMAN EDMISTON: I think that's the issue relative to the discussion later on.

DR. MANGAIYARKARASI: Yes.

CHAIRMAN EDMISTON: Well, thank you very much. Our final presenter is Dr. Prusiner. And, again, would you identify your affiliations?

DR. PRUSINER: Yes, I'm Stan Prusiner. It's up on the slides, the next slide. So I'm from the University of California, where I'm a Professor in Neurology and Biochemistry, and Virology, and I founded a small company called InPro Biotechnology to commercialize some of the inventions that are held by the University of California five years ago. Next slide.

So what I'd like to do is to make short presentation of what I think are some of the problems with prion inactivation, and how we've been able to develop a novel strategy. So prions, of course, are infectious proteins, while viruses are composed of nucleic acid genomes surrounded by a protein coat. Prions resist inactivation by procedures that readily inactivate viruses. And prion diseases, as you know, are invariably fatal with incubation times ranging from one and a half to over 40 years. It's my belief that no exposure to prions should be considered

acceptable. Next slide.

The prion diseases come in three forms, the sporadic form, which is the most common; the inherited form, which represents about 10 percent of all cases, and then the infectious form, which is about 1 percent of all cases. We're really talking about the infectious or iatrogenic forms, the next slide, where we know of cases caused by improperly sterilized neurosurgical instruments, depth electrodes, we know about corneal transplants, dura mater graphs, growth hormone, human gonadotropin, and most recently blood transfusion. The data is not very hard because there's so few cases with blood transfusion, but it looks as though prions have been transmitted in the UK. The next slide.

Prions resist inactivation, and we think that the reason they're so resistant to inactivation is that the infectious particle is very small. There are two lines of evidence that suggest that the infectious particle is a trimer of PrP scrapie molecules. Now when we try to inactivate prions, we can't use procedures that target the genomes of

viruses, bacteria, parasites, spongi, we have to target PrP scrapie, which in itself seems to be a very stable molecule because it has a high beta sheet content. The next slide.

The evidence that PrP scrapie, the smallest infectious unit of PrP scrapie molecules; in other words, the infectious monomer may be a trimer of PrP scrapie molecules comes from two lines of evidence, on of which is shown here. When we do electron crystallography, what we see are these two dimensional crystals, and these crystals through image processing give us a trimeric arrangement. There's a lot of published data that argues that within the unit cell, PrP scrapie is organized as shown here, where one, the first helix, one of the three helixes, and half of the second helix, Helix B, refold into a beta helix, as shown here. These are the unlinked carbohydrates. This is Helix C in the C terminus of the molecule.

Now the other line of evidence that suggests a trimer is the ionizing radiation target data, which suggests that the infectious monomer has a

size of about 60,000 daltons, which would be three PrP scrapie molecules. The next slide. Just press the slide to advance one. Thank you.

In experimental studies, we've approached this two ways. And you've already heard about these approaches using suspensions, which are brain homogenates that offer high titers and a maximum sensitivity range. We've used Syrian hamsters with SC237 prions, and we've also used transgenic mice, where we've studied SC237 prions, as well as human prions. And then we've used the coated wire approach that was developed by Charles Weissmann, and you heard earlier a little bit about this. This shows you the wire implanted into the brain of the mouse. Next slide.

When we look at prions in rodent models, if we transmit Syrian hamster prions into an FEB or any non-transgenic mouse, what is produced in that mouse are mouse prions, not hamster prions. Some people call it a Syrian hamster strain of prions that comes out, but these are mouse prions. They have mouse PrP. If, on the other hand, we knock out the

mouse PrP gene, and now transmit Syrian hamsters into a mouse in which the mouse PrP gene has been knocked out or oblated, and a Syrian hamster PrP gene is now expressed in that animal, we make Syrian hamster prions. Same biology is true for human prions.

Human prions into a non-transgenic mouse produces mouse prions. Human prions into a humanized mouse, the PrP gene of the mouse has been knocked out, human PrP is expressed, produces human prions. And this is very important in bio assays because there is a large lag time or incubation period when you try to innoculate, or when you do innoculate human prions into a non-transgenic mouse. But on the second passage, the incubation time comes down and it remains the same with the third, fourth, and fifth passages, but now you're passaging mouse prions into mice. Human prions into a humanized mouse, the second, third, fourth, and fifth passages all have the same incubation time. The next slide.

We approached assays of human prions first using humanized mice, and then using mice in which there's a chimeric human mouse PrP gene. So, you see,

some of the initial studies, we had to knock out the mouse PrP gene in order for the human PrP to act as an indicator for the transmission of prions. Now the incubation time drops from 700 days to 260 days, and all of the animals become ill.

If we use a chimeric PrP gene, what we see is that it doesn't matter if mouse PrP is expressed. We still have 100 percent of the animals ill, and we see only a very minor drop, probably not really significant if we knock out the mouse PrP gene. To get this further down, we carried out more research because we want to have the incubation times as short as possible, where we reverted two of the human residues, the mouse in this chimeric PrP, and now you see the incubation time is 100 days; with an implanted wire using this mouse model it's about 200 days. The next slide.

We realize that the wire is not a perfect model, but it does mimic the surface of many stainless steel and surgical instruments. And although wires do not reproduce all the shapes and crevices of the hundreds of surgical instruments currently in use,

they do seem to be superior to brain homogenates as a model for these instruments.

Our studies have revealed that there are substantial differences in the resistance to inactivation of human prions bound to steel wires compared to those in brain homogenates, not a surprise. Next slide.

The resistance of human prions to inactivation compared to hamster prions was a surprise. We carried out a series of autoclaving studies, and you'll hear more about this from Kurt Giles when he speaks a little later, he'll give you some of the details of the data. We then bio assayed sporadic CJD prions, the most common form, the MM-1 type in these transgenic animals with these two reversions, and we compared those to the Syrian hamster prions. And what we found is that the sporadic CJD prions in human brain were ten to the five times more resistant to inactivation than hamster prions. Next slide.

In older studies, my view is that -- I have a slightly different view of how to inactivate

prions than the CDC, or WHO. We've commonly used two normal sodium hydroxide for one hour at room temperature to destroy hamster prions but, of course, NAOH is corrosive, as you well know. We found that we need to autoclave for five hours at 134 degrees to inactivate all hamster prions. In the steel wire experiments, we found that 15, 30, or even 120 minutes at 134 degrees did not inactivate all human prions bound to steel wires. And denaturing or hydrolyzing PrP scrape inactivates prion infectivity, but eliminating low levels of prions is generally problematic. The next slide.

Now we stumbled into a novel approach that I'd like to tell you about in the remaining minute and a half. Branch polyamines or dendrimers were found to inactivate prions in the presence of weak acids as room temperature. Even better, SDS in the presence of weak acid inactivates prions. And SDA, of course, is a protein denaturant and a detergent and, of course, it is the most potent of the denaturing detergents.

Residual human prion infectivity, so we've eliminated 99.99 percent, but the residual infectivity

in homogenates down to wires could be eliminated by exposure to acidic SDS combined with autoclaving. The next slide.

This shows the initial experiments that we were surprised when we saw that at pH of four, the proteinase resistant PrP would be virtually eliminated, and at three it's completed gone in this Western Blot. The next slide.

We then substituted SDS, and you see that at these acidic pHs, the PrP is virtually gone. And it doesn't matter what acid we used, whether it's acidic acid, peracetic acid, or glycine, it still had the same effect. The next slide.

So in summary, the dendrimers or SDS combined with weak acids attack an unidentified vulnerable site in the PrP scrapie. Denaturation of PrP scrapie by acidic SDS resulted in inactivation of prion infectivity. The vast majority of the prions were inactivated by acidic SDS at room temperature. Combing acidic SDS with autoclaving for as little as 15 minutes eliminated prion infectivity. Acid SDS may find application as a non-corrosive disinfective, is

capable of eliminating prions from surgical instruments, invasive diagnostic equipment, opthamalogic equipment, and dental equipment. Thank you.

CHAIRMAN EDMISTON: Thank you. Are there any questions from the panel for Dr. Prusiner? Yes, Dr. Jarvis.

DR. JARVIS: What was the source of your PrP scrapie that you used?

DR. PRUSINER: All right. So in the hamster experiments that came from Dick Marsh, who was the first with Richard Kimberlin, working together to take prions that initially started with sheep, and had been passaged into rats, and then into hamsters, so that's the hamster inoculum. The other inoculum was a case of sporadic CJD at UCSF, the human one.

CHAIRMAN EDMISTON: Dr. Grammar.

DR. GRAMMAR: In your bio assay for infectivity, can you explain that?

DR. PRUSINER: Sure. So what we do is in homogenates, if an undiluted sample in an end point titration represents a 1 percent brain homogenate,

because you can't innoculate a 10 percent brain homogenate, and we inoculate 30 microliters of it into the thalamus, the region of the thalamus, and we do this in four animals, eight animals, depending on how many animals we're going to use at any dilution. Then you can do serial ten-fold dilutions.

One of the problems is that prions, as you've heard, clump. They aggregate, and so you're dealing with a suspension, you're never dealing with a solution. And you're going to get an end point which is imperfect, but even in viral assays, on lawns of bacteria, on lawns of mammalian cells, you get an end point which is imperfect; meaning, you don't get all, it's all or none in the next dilution. So it's a typical end point titration assay. That's for homogenates.

For the wires, we don't know how to do. We don't know how to quantify the number of prions in the wire. We don't know how to get the prions off the wire. I'm not sure that you can simply do dilutions. There's been one paper published, the one you heard about in the Lancet, where they tried to do dilutions,

and then take each dilution and add that -- and immerse the wire in it, and then dry the wire, and then implant the wire. And you don't get very good curves of doing that. We have not spent time doing that. We may do that in the future, so that's how we assay.

Now we've inoculated the animals. We now wait. And then we check the animals twice a week. And when the animals begin to develop neurologic signs, we watch for a progression of the disease. A small number of animals we will necropsy, but we watch this progression of the disease so this is a progressive neurologic disease, and then we sacrifice the animals just before death.

DR. GRAMMAR: In your Western Blots, where did you get your antibodies?

DR. PRUSINER: We made them.

DR. GRAMMAR: You made them? So they're polyclonal, or monoclonal, or what?

DR. PRUSINER: They're monoclonal and recombinant fabs. We purified -- we identified the prion protein 25 years ago, and we took the purified

protein and we made antibodies to it, first polyclonal antibodies, and then since then we've made numerous monoclonals, and even recombinant fabs.

DR. GRAMMAR: What'S the limit of sensitivity? Like how nanograms or picograms, or whatever can you detect with your antibodies?

DR. PRUSINER: Well, it depends on the immuno assay. We can detect --

DR. GRAMMAR: What's the best immuno assay you have?

DR. PRUSINER: I'm not sure I want to give you that number right now. I'll give it to you later.

DR. GRAMMAR: Okay. Well, forget about that.

DR. PRUSINER: Okay.

DR. GRAMMAR: Can you get down to pico moles, can you get down to phenta moles?

DR. PRUSINER: Yes, we can get down to pico moles.

DR. GRAMMAR: Not phenta moles.

DR. PRUSINER: No.

DR. GRAMMAR: Thank you.

CHAIRMAN EDMISTON: Dr. Jarvis.

DR. JARVIS: Do you have any evidence from other sporadic human CJD prions that they would have different susceptibility or resistance to your treatment?

DR. PRUSINER: No, but certainly different strains have different susceptibilities. And, you know, this is the beginning, this work is really the beginning of looking at human prions, so one could envision extremely large numbers of studies to try to reproduce what is in animals. And some strains may be more resistant than others, but we have not seen any strain of prion that resists acidic SDS.

DR. JARVIS: What is your feeling about extrapolating from scrapie versus using sporadic CJD from humans for these types of studies?

DR. PRUSINER: Well, I think there's a problem, because we were surprised to find that the prions of human were ten to the five times more resistant than the standard scrapie hamster model. I think you want to use human prions now that the models are available. And particularly since we have one

that's short enough now, 100 days.

DR. JARVIS: Are these models proprietary or are they widely available that you're describing?

DR. PRUSINER: They're proprietary but they're available.

DR. GRAMMAR: Can you buy them from Jackson Labs?

DR. PRUSINER: No.

DR. GRAMMAR: Okay.

CHAIRMAN EDMISTON: Dr. Coffey.

DR. COFFEY: Yes. So if you're not inoculating or using steel wires in these studies, what exactly are you treating? Where are you administering the treatment? Is it to the diluted allo quats, brain homogenate?

DR. PRUSINER: No. We're doing the undiluted brain homogenate, 10 percent brain homogenate.

DR. COFFEY: Okay.

DR. PRUSINER: And then we dilute that.

DR. COFFEY: Right.

DR. PRUSINER: We have to dilute that

about 100-fold. So if we take a brain homogenate, and we simply want to measure the number of prions in it, we can dilute it ten-fold, and we can get 100-day incubation period. If we want to mix, for instance, acidic SDS with it, we now dilute it 100-fold, so we end up with a .1 percent brain homogenate, which is the most potent we can inoculate into the animal because we have to dilute out the SDS and acidic acid.

DR. COFFEY: So the SDS is added to various serial dilutions of the brain homogenate?

DR. PRUSINER: No, no, no. We add the SDS to the 10 percent brain homogenate. We do the inactivation, and then after that, we then would do a series of dilutions just before we'd inoculate it in animals.

DR. COFFEY: Okay.

CHAIRMAN EDMISTON: Dr. Arduino.

DR. ARDUINO: So you're not actually doing your little stainless steel rods, or have you inactivated --

DR. PRUSINER: Yes. Yes.

DR. ARDUINO: Okay.

DR. PRUSINER: We've put that in acidic SDS and done those studies, too.

DR. ARDUINO: Okay.

CHAIRMAN EDMISTON: Let me ask you a question, because you're aware of the differences. We passed around some examples of bio materials, the stainless steel pins, and an example of a hemostat.

DR. PRUSINER: Yes.

CHAIRMAN EDMISTON: How relevant do you feel is the model system in terms of the simulation from clinical relevance perspective? In other words, if you look at a hemostat, if you look at hinged devices, you look at devices that may be composed of multiple substrates, the titanium with the Teflon sealer, how relevant should that be in terms of trying to validate these models?

DR. PRUSINER: Well, I think we're at the beginning of all of this. We're not 10 years down the road where there's already ways to inactivate, and we're trying to make it incrementally better.

One of the problems you have in making kinks in the wire, or bending the wire and turning it

into a U, or making a loop in the wire, is that you change the contact with the brain, and you make it very irregular.

The beauty of the model that Charles Weissmann settled on is that the entire wire is in contact with the brain, and you leave it there. So, as you heard earlier, you get the maximum sensitivity doing that. And it is thought from his studies that the prions that are bound to the wire are contacting PrPC on the surface of cells that are in contact with the wire, acting as a template for PrPC, which is now getting converted. So if you prevent the PrPC on the surface of the cells that are supposed to be touching the wire from touching it, you won't get this conversion to occur. It's not as though the prions are being liberated from the wire as it sits there in the brain, and going all over the brain. Sure, new prions are being formed, but those are initiated by the prions on the wire. So I don't know of a better system at the moment.

Now Weissmann has done some studies where he's not published much of the details with plastics,

and he didn't see much difference between plastic and stainless steel, but there are not many details in the literature. And we just heard a moment ago, the first presentation about plastic and stainless steel. We've not done any work with plastics.

There are an infinite number of stainless steel grades that you could look at. There are an infinite number of other alloys, other metals you could look at, and plastics. And just where you start and where you stop is not clear to me. I think this is a model that's been developed first by Charles Weissmann and a few other groups, and our own group, and we are getting reproducible, I think reliable data from it.

Now can we expand this? Of course. It can be bigger, and bigger, and bigger, but I think at the moment, this is a very good model of prions bound to a surface.

CHAIRMAN EDMISTON: Are there any further questions? Yes, Dr. Jarvis.

DR. JARVIS: If you use the wire, and instead of leaving it in contact for a year, insert it

in the brain and leave it for one hour, two hours, five hours, and then take it out; how does that change the model?

DR. PRUSINER: Weissmann has done that, and the animals get sick. It may prolong the incubation period a little bit, but they tend to get sick. I mean, he's done another set of experiments where he puts the wire into a brain of an animal that he's just sacrificed, pulls the wire out and sticks it into the brain of the animals. He claims from -- I should say his data state that those are even a little more infectious than the wires where they have been immersed in a homogenate for two hours, then air dried overnight, and then inserted into the brain.

I don't know how we could do that experiment. We'd have to be hovering around the CJD patient, waiting for the patient to die, then remove the brain, or at least open the cranium and stick the wires in, then stick them in the animal, so that's not something I want to do. So from our point of view, the best approximation is to take frozen CJD brain, make a homogenate from a piece of it, takes the wires,

immerse them, air dry them, and then insert them. I don't know how to do that experiment better in a practical, meaningful way, and leave the wires in indefinitely so that we have the maximum contact.

CHAIRMAN EDMISTON: Any further questions? Thank you very much. At this time, I'm going to modify the schedule a little bit. Rather than take our break, I thought we'd move right on to the second open public hearing. We have one speaker scheduled. At that time, I would also, after that speaker makes his presentation, I'll ask members of the panel are there any additional questions they may like to ask members of the audience who have already previously presented. Could I have Mr. Kurt Giles? And as per the usual rules, we're limiting the presentations to ten minutes.

DR. GILES: Hello. My name is Kurt Giles. I'm an Assistant Professor at the University of California San Francisco. I've had 15 years experience working in neuro degenerative diseases. I've previously held faculty positions at the Weissmann Institute of Science in Israel and at Oxford

University. For the past three years, I've been directing the transgenic research at the Institute of Neuro Degenerative Diseases at the University of California. I also head the Prion Inactivation Project there. I also have a financial interest in Pro Biotechnology.

The current recommendations for inactivating CJD prions are based either on hamster prions or on passaged CJD prions. As you heard in Dr. Prusiner's presentation, a CJD prion which is passaged in a mouse or in a guinea pig is no longer a human CJD prion. It is a mouse prion. It's a mouse protein or a guinea pig protein. You wouldn't necessarily assume that a mouse protein or a guinea pig protein behaves like a human protein. So that was really our basis for doing these wide range of experiments. We wanted to have a look at in activation on human prions, as well as inactivation of other prion strains. Next slide, please.

So to look at these different prion strains, we wanted to use the most sensitive models available, and fortunately, at the Institute of Neuro

Degenerative Disease, we have the most sensitive models available. And these are a transgenic line of mice expressing hamster PrP, which are extremely susceptible to the Sc237 strain, equivalent to the 263K hamster strain that's been mentioned in other presentations.

We also have a line of transgenic mice that are extremely sensitive to human CJD prions, and these range of mice actually express a chimeric mouse/human PrP. We tested both infectious brain homogenates and the wire model that you've heard from a few presentations, and then we assayed the samples before and after treatments with acidic SDS.

As you heard in the presentations this morning, the way that you want to be sure of how much infectivity you've got rid of is that you need to understand the relationship between the titer of the infectious agent, the proportion of animals succumbing to disease, and the incubation period of these animals. And then you want to take the step of comparing your inactivation protocols against these standards. And we use survival analysis techniques

for all of this, which is -- it gives a lot more statistical and scientific rigor than sort of the simplistic analyses that have been performed before, just looking at mean incubation periods.

So the next slide. This actually shows a technique for doing exactly what I described on the previous slide, and this is essentially survival analysis. It's a very common technique in human treatments and drug testing. Essentially, you're looking at the length of time that an animal survives, so what we have here, on this axis we have the proportion surviving, and then each of these lines represents one of the serial dilutions. So at a ten to the one dilution, a 10 percent brain homogenate, all the animals become sick with immediate incubation period here of about 45 days.

As we dilute further and further out, the incubation time increases, and as each of the animals die, and you're also seeing on this graph the proportion of animals that are dying from a particular treatment here, because after this dilution, the ten to the minus eight dilution, you see all the animals

are still succumbing to disease. At the ten to the minus nine dilution, again, the incubation period is further extended, and not all animals succumb to disease. As you dilute further and further, you get to a point here in this place, it was ten to the minus eleven dilution, where no animals succumb to disease. And that is then the limit of the sensitivity of the assay. Next slide.

So what we found rather surprising was that nearly every treatment we tried with acidic SDS completely inactivated hamster prions, but the human prions were invariably more difficult to inactivate. Next slide. So I'm presenting some data here, so this is an example of using an acidic SDS treatment for 30 minutes at 65 degrees; we're comparing positive controls with treated. So in addition to doing the serial dilutions that I showed, which sort of form the basis, and we did those both for hamster prions and for human prions, for each of our experiments we then have essentially a positive control, and a treative group. So in this, one of our most mild treatments, we nearly inactivated all the prions, but we still -

because we're looking at median incubation period here, so you only get a median incubation period when half the animal die, so here we actually have less than half the animals dying so we have no median incubation period. But what these means is that we still have a quantifiable reduction, and that's very important.

In some of the presentations you've seen earlier, you can't compare no deaths with no deaths because you don't know the limit of the inactivation that you've done. You just know that you've inactivated to the limit. So we specifically chose sub-optimal procedures here, where we didn't complete inactivate. And as you see, so we got a massive reduction in the hamster prion titer, about a nine log reduction, when we did exactly the same treatment on human prions, we got essentially just about a doubling of the incubation period, still the vast majority of animals succumbing to disease, and that comes out at a 3.8 log reduction. So this is extremely important because this is saying that there's a five log difference between these two strains. Well, human

prions are 100,000 times more difficult to inactivate than hamster prions.

Now from presentations that you've heard earlier today, when people are talking about a six log reduction, a six log reduction on hamster prions is equivalent to a one log reduction in human prions. So this was really a surprise to us, but this really shows that if you want to think about procedures to inactive human prions, you've got to look at human prions. Next slide.

The next finding, and this is also being found by others, as well; but the comparison between inactivating brain homogenates and stainless steel wires - this happens to be, I just chose an example from the hamster strain, and a slightly different acidic SDS treatment. So inactivating brain homogenates with this treatment completely inactivates brain homogenates, no more detectible infectivity within the limit of this model.

With the prion coated wires, we extend the incubation period, but you see here nearly 70 percent of the animals are still succumbing to disease, so I

should point out, the data that I'm presenting is a paper that is currently in press in "Journal of Virology".

We then looked at the effect of autoclaving on CJD. Again, all the work that's been mentioned earlier has not been directly on CJD, so in all these standards about sodium hydroxide and things, these weren't even tested on CJD. These were tested on a mouse or a guinea model of CJD. And so all these recommendations that are there for inactivation of CJD are not based on CJD. What we're finding, because we're the first one to report this kind of data directly on CJD, is that these procedures that we thought inactivated prions, don't inactivate human prions. They may inactivate hamster prions.

A fifteen minute treatment at 134 degrees for human CJD prions on the stainless steel wires slightly increases the incubation period, and about three-quarters of the animals succumb to disease. As you see, even two hours autoclaving at 134 degrees, you have nearly half the animals still succumbing to disease. However, we have developed treatments that

will completely inactivate human prions, even those bound to stainless steel wires. Just, again, by comparison, the same treatment, a 15 minute autoclaving treatment, we have three-quarters of the animals dying. Fifteen minutes with acidic SDS, we have none of the animals dying.

So to conclude, we believe that methods for inactivating human prions have to be validated on human prions. The currently recommended methods were recommendations made on the best data available at the time the recommendations were made. We now have better data. These recommendations need to be updated. And to conclude, we have developed methods that can inactivate human prions, even when bound to stainless steel surfaces. Thank you.

CHAIRMAN EDMISTON: Dr. Giles, I'd like to ask you a question, because you made a provocative statement there. And maybe I heard it incorrectly, but you said a six log reduction in hamster prions would be equivalent to a one log reduction in human prions.

DR. GILES: That's what our data shows.

CHAIRMAN EDMISTON: With that statement, and you look at the risk assessment that the FDA developed, were you available to listen to that presentation by Mr. Brown?

DR. GILES: Yes.

CHAIRMAN EDMISTON: If you look at their log reduction infectivity, if that was the case, and if our current cleaning and sterilization procedures were only achieving a one log reduction, wouldn't we have hundreds, if not thousands, of cases of CJD, rather than less than one per million?

DR. GILES: Well, it's --

DR. EDMISTON: Do you see what I'm saying?

DR. GILES: Yes.

CHAIRMAN EDMISTON: If our current sterilization procedures and disinfectant procedures are not effective, and that we're not getting the reduction we really should be getting of human prion, wouldn't that suggest we see epidemiologically more cases?

DR. GILES: It depends how many operations are being performed on people with CJD, and whether

that will be increasing. And there may be a 20 year incubation period, so in 20 years time we may see the epidemiology to show that.

CHAIRMAN EDMISTON: Is Ron in the audience here? Am I saying this correctly, Ron, in terms of your data? You looked at log reduction. In fact, you actually start at four log reduction. You don't have anything --

DR. BROWN: We did, and we assumed species equivalence in the inactivation of the prion. So I was thinking the same question.

CHAIRMAN EDMISTON: So my take on your data would be valid in terms of that premise, that there's only a one log reduction. Then why aren't we seeing more cases out there?

DR. BROWN: I think that's an interesting question.

CHAIRMAN EDMISTON: Yes, Dr. Coffey.

DR. COFFEY: Yes, just to jump in, and maybe Dr. Haines will also jump in. In my experience in neurosurgery, and Steve may have some figures nationwide, only a minority of neurosurgical

procedures are cranial procedures in this country. And only a small minority of those actually involve an instrument penetrating the parenchyma of the brain. And those operations would be those involving an intra-axial or intra-parenchymal tumor, or maybe an epilepsy resection, or perhaps even a stereotactic operation. But those are only a very small proportion of neurosurgical procedures. And of the instruments that were illustrated, that we've seen in various of the talks, it's entirely possible that most of them, or perhaps even none of them, or of a typical 20-instrument kit would even touch the brain, even in a a parenchymal neurosurgical operation. The only thing that touches the brain in many brain tumor operations, for example, is a suction tip, and a bipolar cautery. So that may be responsible for the fact that even though nothing works, we're not seeing an epidemic.

CHAIRMAN EDMISTON: This is a totally confusing discussion, and I'll tell you why. On one hand, you present an argument that we're not doing the right thing. On the other hand, we have data available, be it statistical data projections, that

the risk is low. On the other hand, you point out that because of the limited number of surgical procedures in which there's actually patients at risk, it's really hard to fathom how important this whole issue is, to be perfectly honest with you. I mean, it's an emotional issue, but the point is, what is the true risk, and at what level should we be concerned?

DR. GILES: So if you take the precautionary approach --

DR. EDMISTON: Yes, that sounds great, but the point is, I'm just concerned by your statement, which is highly provocative, which may be correct, that, obviously, if that is a correct statement, we are missing the majority of CJD patients that are coming down the pike. That's what your data would suggest. Dr. Haines.

DR. HAINES: Well, if I understand the statistical model correctly, it assumed -- it does not include any screening of these patients, and the deliberate management of operations on patients with known or suspect CJD, to throw away the instruments and reduce the risk immensely by doing that. So I'm

comfortable that this model gives us, within the uncertainties that are included in it, a reasonable idea of where we stand in operations done where we do not suspect that the patient has CJD. But that it doesn't address the direct issue of what if there are patients out there who are asymptomatic carrying infective prions that we don't -- that we can't detect now?

CHAIRMAN EDMISTON: Dr. Jarvis.

DR. JARVIS: I think probably one clarification is important. I don't think there is any such thing as a prion carrier. You're either infected with it or you're not. You may have not progressed to disease yet, but you're not a carrier like an MRSA carrier, where you carry it the rest of your life, and die of a heart attack, and have no evidence of disease.

DR. HAINES: On an infected person who is yet undetected.

DR. JARVIS: Right. I think it fails to take into account a very important part of clinical medicine, which is any device used in the operating

room goes down, and is disinfected first. What would happen in your model if when you take that wire, put it in the homogenate, let is dry, then you put it an instrumatic cleaner for an hour, and then put it into the animal, my guess is that the infectivity would be tremendously reduced. And that's not included in any of these models.

DR. GILES: There has been a published study again done on rodent prions; and yes, it did reduce something like a four log reduction on rodent prions in a transgenic mouse model. What that does on human prions, it hasn't been tested, as far as I'm aware.

DR. JARVIS: So that could be nine right there, nine log difference.

CHAIRMAN EDMISTON: Dr. Priola.

DR. PRIOLA: I was wondering if you could clarify for me experimentally a couple of the things that you did. All the data you show for sporadic CJD brain, this was all from the same brain homogenate. Right? Everything --

DR. GILES: It was from the same patient.

There's about 100 different experiments listed in that paper, and they're from the same patient, two preparations made, I believe.

DR. PRIOLA: Okay. And how did you -- so it gets back to Dr. Jarvis' question. I think you were the one who asked it; how many -- you've got a sample of one for you sporadic CJD data. And it's very possible that if you were to test more brains, you would start to see a range, as opposed to assuming every sporadic CJD strain is 100,000 times more resistant to inactivation than a rodent strain.

DR. GILES: Well, you're right. We haven't tested more than this one human strain with this method, although we have -- well, we've tested other prion strains that -- are you saying that there's individual variation between human, or possible --

DR. PRIOLA: Yes, I think it's -- I'm sure there is. I'm sure there.

DR. GILES: And would you expect the same variation between hamsters --

DR. PRIOLA: Well, the hamster situation

is very different because that strain has been passaged for a very long time in the laboratory, and it has been specifically adapted to the hamster, so that's a little bit different issue. I guess all I'm wondering is if there are experimental parameters that might help to explain this 100,000-fold difference, and that's just one of them that you have essentially.

A second possibility is -- well, how did you determine the titer of the strain? Did you determine the titer in the same animal that you did the experiments in?

DR. GILES: Exactly, yes. Yes.

DR. PRIOLA: And one thing, and I know it's not usually an issue. You can really store brain homogenates for a very long time and not loose any appreciable level of infectivity, but the physical state of the brain homogenates could conceivably be very different. I mean, if you're taking a frozen section of perhaps an older sample of sporadic CJD brain and comparing it to --

DR. GILES: So we have been using this brain over a period of 10 years, and dozens of

experiments that all end up with very equivalent incubation periods.

DR. PRIOLA: So it does make me sort of wonder, and I understand it's not something that you can easily test, or you can begin to approach that over that 10 year period where you're taking this material, going back to this brain time and time again, are you dehydrating it?

DR. GILES: Well, we're always getting the same amount of infectivity from it each time we take it.

DR. PRIOLA: Okay. Well, that's -- okay. Thank you.

CHAIRMAN EDMISTON: Are there any further questions for Dr. Giles? Well, thank you very much. Do we have any additional public presenters? At this time I'd like to ask the panel if there are any questions that they would like to ask, that they wanted to ask but didn't get a chance to ask earlier in this session to any of the members of the audience who have made presentations. Yes, Dr. Haines.

DR. HAINES: I have one, and I don't know

quite who to ask it of. But when the instruments are placed in an ultrasonic or enzymatic cleaner, a large number of instruments are placed in the device, is there any concern about cross-contamination from an instrument that has been in an infected brain, to an instrument that was used on someone else?

DR. MURPHEY: Well, if you had an instrument from a patient whom you knew had CJD, or suspected had CJD, you would not, initially at least, process it in the general manner with other instruments. I think the risk would arise if you have an instrument from a patient whose risk has not been recognized.

The potential for cross-contamination of the other instruments in an automated cleaning system I don't think has really been very carefully examined. These machines use large volumes of heated water, large volumes of detergent enzymatic cleaners, so that you're mechanically removing soil over time.

The relative efficiency of that in terms of cleaning them seems to be fairly good, particularly when you look at other pathogens that we would worry

about in a hospital. For instance, the instruments used in a patient who has a serious MRSA infection, we're going to send them downstairs to central processing. They're going to go through the routine processes that an instrument used on a patient who didn't have an infection at all would go through. Now we feel very comfortable that when those instruments are sterilized, the sterilization processes, or even high level disinfection if we're talking about endoscopy is, in fact, going to take care of any remaining residual inoculum. So the level of anxiety would be very different from the level of anxiety that we would be dealing with with prions.

DR. COFFEY: Just a follow-up question.

CHAIRMAN EDMISTON: Dr. Coffey.

DR. COFFEY: Yes. Thank you. What happens to the liquid medical waste from these various processes? I mean, I know that sounds like a naive question, and maybe someone from industry should answer it.

DR. MURPHEY: It's not a naive question. It would be true for both liquid and solid waste.

There are infectious materials in there. Normally, infectious waste is treated by diluting it and putting it out in the liquid waste stream. This is what we routinely do for Hepatitis-containing fluids, HIV-containing fluids, and we also do it for potentially prion contaminating fluids.

Now, in fact, the volume of truly fluid material that would be disposed of in that manner would be very small, and it would be extremely diluted in the waste stream. And then you would have to ask yourself, well, what's the possibility that someone would have an effectively transmissible encounter with that waste stream material. And the answer is very small, but of course, not zero. We would worry most about a sharp, such as a needle, a spinal tap needle or something, that was immediately contaminated with blood or spinal fluid, then sticking a healthcare worker. And there are such episodes reported in the literature with follow-up. Exactly how much follow-up depends on the exposure, and we are not aware of any reported cases resulting from such exposures; which, again, if you don't know the efficacy of the follow-

up, does not truly answer the question.

But once something gets flushed out into the sewerage system, which is, in fact, what happens much of the time, we think the material would be so diluted and the other systems that would usually be applied to liquid waste would probably reduce the inoculum sufficiently that we don't have to worry about it. We're not aware of any transmission by that route. And the same thing would be true for solid waste.

CHAIRMAN EDMISTON: Well, in research we had an old adage, the solution to the pollution is dilution, and that's how we go through our lives every single day. Can you stay up there for a moment, please?

DR. MURPHEY: Sure.

CHAIRMAN EDMISTON: I'd like to ask Dr. Favero if he could step to the podium, please. Now, Dr. Favero, you have over 20 plus years. I hesitate to guess exactly how many, of dedicated public service. And I know you are in industry, but I want you to take off your industry hat now and give us a

little perspective as an infection control person in terms of the current infection control perspective, albeit APIC and CDC on the log reduction or the reduction of infected particles as it applies to prions. I know you don't have any slides, but I know you can do it.

DR. FAVERO: Thank you very much. Dr. Edmiston. You will be amply rewarded for this.

(Laughter.)

DR. FAVERO: My name is Martin Favero. I'm the Director of Scientific Affairs for Advanced Sterilization Products, which is a Johnson & Johnson company. We also are very, like our colleagues at STERIS, interested in methods for sterilization, and in particular, to inactivate prions, and we've also funded studies that have been published in the literature.

I'd first of all like to thank this panel and you, Dr. Edmiston, and especially the FDA for this excellent meeting. This is the best review of the problems put on by the government that I have ever attended, so I really congratulate you for that.

To answer your questions, I sort of agree with one of your last comments, and that is what really is the risk, since we have no reported cases. But on the other hand, I realize that a lot of our colleagues in hospitals, in the surgical profession keep asking the question, what can we do?

I first got interested in this back in 1995 when I was at CDC, and realized we did not have any guidelines for prions or CJD. The only thing we have, Bill Jarvis had written a very nice chapter in one of the infection control books, but he, like all of us, was in the position of quoting the prion scientists relative to what methods there were to inactivate prions. And as you've heard repeatedly today, virtually all of those methods are not extrapolatable over to our current situation.

The thing that encourages me is that in the last year and a half there have been a number of publications that have sort of married prion science and sterilization sciences together, and we're starting to see some very nice publications on inactivation of prions.

What I would suggest, I'm not sure if your entire panel, Chuck, has seen all six or seven of those publications. If they have not, they ought to. What I am discouraged at is that we still seem to be making a mountain out of a molehill. I thought that for years. I would encourage my colleagues at CDC, I understand their position, and the position is very simple; is that, when they come out with their guidelines, they have to be politically correct, and include the WHO recommendations, some of which are actually totally preposterous, to be frank.

One would never place hot sodium hydroxide in an autoclave. It's a hazard. Now why we can't say that is beyond me. Now Dr. Schonberger pointed out that there are some sort of signal recommendations in CDC guidelines. In some context we call them Category 2, which means maybe you can do this if you want, or as he pointed out, you might have a phrase like if it's not feasible or cost-effective, you can use something else. And that is a key for the hospital personnel to pick up on, but sometimes they don't pick up on it, and so then you have situations as Dr. Burke

has mentioned, where you have a hospital destroying $12 million worth of instruments. So I guess what I'm saying is I hope my colleagues will be less politically correct in the future.

I think the only other thing that I have is I'd like to ask a question, since you got me up here; and that is to our colleague from the UK, or anyone else, to comment on Dr. Helen Baxter's recent paper in the "Journal of Virology", not on the results of the plasma, because the plasma system that etches is not going to be compatible with medical devices, but with the experimental procedure of inoculating stainless steel spheres and placing them in the peritoneal cavity. I haven't heard that discussed as whether that's a good, bad, or indifferent method.

CHAIRMAN EDMISTON: Does anybody wish to comment on that?

DR. BURKE: I have a comment on the washing.

CHAIRMAN EDMISTON: Could you identify yourself again, please.

DR. BURKE: Yes. Dr. Burke with STERIS

Corporation. You had asked a question on washing. If you go back to the Lancet paper that we published in 2004, we did studies on serial dilutions up to ten to the fifth, from zero to ten to the fifth, and simulated the best we could under those conditions moderate washing conditions that would be seen in a hospital. What we were very surprised with was the infectivity level we theorized due to the hydrophobic nature of the material was exactly the same, so mean dead. And instruments with scrapie was the same, so washing as a means of eliminating the prion molecule, and I think Dr. Prusiner talked about this, as well, is probably not as viable as many people think it is. It needs some other type of treatment.

CHAIRMAN EDMISTON: Yes.

DR. GILES: I'd like to address the question you had about the stainless steel --

DR. EDMISTON: Please identify yourself, again.

DR. GILES: Kurt Giles, University of California. So stainless steel spheres were, as I understand the experiments, were implanted into the

peritoneal cavity of hamsters. The amount of infectivity you can measure this way is a lot less than via intercerebral inoculation, because the animals don't get sick as quickly when inoculated through the stainless steel sphere intraperitoneally, and so, therefore, you've got a much smaller range over which to measure inactivation. Being the hamster model, it only works with the hamster prion strain, as well, and this kind of experiment wouldn't be possible in our transgenic models because they don't absolutely mimic the same natural level of peripheral inactivation. So it's an interesting other model, but it's very limited in both the strain it can look at, and the range of inactivation you can look at.

CHAIRMAN EDMISTON: Are there any further questions for the panel? Dr. Telling.

DR. TELLING: Dr. Prusiner, or Dr. Giles, actually. So we've been asked to make statements or to assess whether the validation studies in animal models are really a reasonable approach for companies to claim reduction of TSE infectivity. And also, discussions have been aired today about various

immunologic approaches for detecting PrP.

I know that you've been developing over the years immunological approaches for detecting infectious forms of PrP, CDI in particular, and that you've shown in previous publications that those assays are at least as sensitive as the animal transmission studies. I was wondering, and I realize that these are early days, but whether you've addressed t his directly with your inactivation studies, comparing the animal transmission data with the CDI?

DR. PRUSINER: No, we've not done this with the CDI. What we have done is done a lot of Western Blot studies, and I think from my point of view, it's very important to do these immuno assays, because then you know you're on the right track, or you know you're not going down a good track. But in the end, I think you want to know the infectivity. And I don't think that a CDI is going to substitute for knowing the infectivity, and so that's why we didn't carry out a whole series of studies for the CDI test, even though I think at the moment, the CDI test

is probably about 50-fold more sensitive than the IO assay. And this comes back to the definition of an infectious unit that you were having a discussion with one of the other presenters.

In reality, an infectious unit is about a million PrP scrapie molecules. But as soon as you inoculate this into the brain, most of that's gone, and you end up with about 1,000 PrP scrapie molecules per infectious unit. This is a paper we published very recently in the "Journal of Virology." So I think you're always begging the question when you do an immuno assay. You want to do immuno assay so that you know you lay out all the experiments, you get a very good idea of what's going on. And it's not to minimize the importance of immuno assays, as very quick and very, very useful, but I think in the end, you want to know the number of infectious units that persist.

CHAIRMAN EDMISTON: Thank you. Any further questions? I think at this time we'll take a break. I have about quarter of three. We'll convene a few minutes after three o'clock, and we'll begin the

deliberation of the panel questions.

(Whereupon, the proceedings went off the record at 2:47:55 p.m. and went back on the record at 3:04:09 p.m.)

DR. JARVIS: May I ask a clarifying question before you go to the questions? I guess a question for me and for our deliberations, if I look at the background information, it specifically says that we should be focusing on CJD, and I guess two clarifications. One, does that include variant CJD, or is it just sporadic CJD? And secondly, if that's the case, then don't we have to be somewhat skeptical, or at least question data that comes from scrapie Kuru, BSE, et cetera, et cetera, that isn't really CJD?

CHAIRMAN EDMISTON: That's a good question, and I think that rather than give you an answer right now, let's address it as we go through the various questions.

At this time, could we get the FDA to read the questions?

DR. MURPHEY: "Members of the panel, we

are asking your advice on the following questions. We apologize in advance for the fact that they're very difficult. You've heard a lot of very good testimony today, which I think explicates very nicely the problems which this field brings to the table.

Question number one - assuming that a product sponsor seeks a claim for reducing TSE infectivity on stainless steel instruments, is it reasonable for such an indication to be validated using animal studies of TSE transmission? Please discuss. Second - discuss the relevance of various design features of such validation studies. Third, of the three study end-points cited in the literature, log reduction in infectivity, mean incubation time and survival as median survival or percent survival, which, if any, may be adequate for the validation of a reducing TSE infectivity indication? Should demonstration of a particular level of reduction of TSE infectivity in one or more end-point be expected in order to support an indication for use? How may clinical benefit be estimated from these end-points?

Fourth - what additional issues should be

considered by FDA when evaluating indications for use for devices other than simple stainless surgical steel instruments? How can devices constructed from or including materials other than stainless steel, devices with complex shapes, devices with hinged or mated surfaces, or devices with lumens be addressed? How closely should the experimental treatment conditions for a product or process indicating to reduce TSE infectivity replicate the actual conditions under which the proposed product or process would actually be used? Should such issues as instrument cleaning conditions which might fix proteins to instruments, possible interactions between a new product or process and standard cleaning agents, sterilizer cycles used, et cetera, be considered? And finally, considering the current state of the science, and existing investigative methods for estimating the potential for TSE transmission, can an indication for use of complete reduction of TSE infectivity, complete elimination of TSE infectivity be validated? Thank you."

CHAIRMAN EDMISTON: Dr. Murphey, before

you sit down, could I ask you a clarifying question?

DR. MURPHEY: Certainly.

CHAIRMAN EDMISTON: Do you wish this panel to deliberate primarily on CJD, or should we look at the bigger picture here, as has been suggested by Dr. Jarvis?

DR. MURPHEY: I think I would have to leave that question up to you. In terms of the likelihood of transmission ATSE by surgical instruments in the United States, sporadic CJD would be the most likely candidate for consideration. However, if you want to consider the worldwide implications of your discussion, you should certainly include variant CJD, and potentially the genetic forms of TSE, as well.

CHAIRMAN EDMISTON: Dr. Jarvis.

DR. JARVIS: I guess I would just raise the issue that in terms of both for us, lack of data, putting the manufacturers through enormous hoops, if we leave this as TSE infectivity, I guess I would demand personally that the tests be done on every single agent that would fit within that category under

the various conditions that we've talked about today, which I think if we do that, I can't imagine any manufacturer will be able to comply with that.

DR. MURPHEY: I would be inclined to agree with you in terms of the feasibility of such studies. And we are asked to consider feasibility when looking at a potential product that is being brought to us, and the actual ability to do validating studies.

CHAIRMAN EDMISTON: Could we go to the first question again? Does that panel have any comment? Yes, Dr. Schonberger.

DR. SCHONBERGER: As an issue relevant to whether vCJD is something that's pertinent to the group; clearly, classic CJD is overwhelmingly what we're seeing, but people should not assume that vCJD is not in our hospitals at all, because we've had the patient from Florida went to a couple of hospitals. My understanding is that we know we have had cases in San Francisco, I assume because of the reputation there with Stan Prusiner, that they've had people coming for diagnosis and treatment, I think, at one point, so they've had it there. I think Mayo Clinic

has seen one of the international cases, and even the Saudi Arabian case was in the United States for a period, so there aren't that many variant CJD cases, but we are in the international community, and occasionally they come in, and sometimes -- I don't know of any of them that have actually had neurosurgery, however, here. They usually come in for other types of procedures, but the point is that yes, CJD is key. The regular classic CJD, but we're not totally an island here, not affected by vCJD issue.

CHAIRMAN EDMISTON: Why don't we read that first question again.

DR. SCHONBERGER: Just the question or a comment to clarify. I'm a member of the Department of Defense. I just want to get an idea of what the incidence of CJD is kind of globally. Right now we have forces deployed to Afghanistan and Iraq that are performing neurosurgical procedures on local nationals. Those instruments are being maintained in those facilities, probably with less sterilization capability than we currently are using state-side, so if somebody could clarify that question for me as we

go on to deliberation.

CHAIRMAN EDMISTON: Dr. Murphey.

DR. MURPHEY: What data we have, although it is limited from some parts of the world, suggests that the estimated prevalence in terms of deaths from CJD really does average about one per million worldwide.

CHAIRMAN EDMISTON: "Assuming that a product sponsor seeks a claim for reducing TSE infectivity on stainless steel instruments, is it reasonable for such a claim to be validated using animal studies of TSE transmission?" Any comments? Yes, Dr. Butcher.

DR. BUTCHER: Well, I would just look at what was presented, and say that we would have to go back and look at all of those studies to see if we're really with the human form as the latter presenters suggested that we're just dealing with truly animal form. And it does present differently.

CHAIRMAN EDMISTON: Dr. Telling.

DR. TELLING: I've written down some thoughts here which might be useful to share. I think

with respect to the validity of animal models, I think Dr. Prusiner put his finger on it. I think since experimental bio assay is the only means of detecting prion infectivity, you really would want to have those studies in place. And because now we have an array o various transgenic models in which we assess prion infectivity from a variety of different species, including human prion infectivity; in addition, we can also assess new variant, or variant CJD infectivity, not necessarily with the humanized mice, but certainly - because it is related to the BSE strain, we can assess infectivity using Bovinized transgenic mice, because it does appear to behave in this respect in the same way as BSE.

There are additional new other promising approaches that are in the pipeline, and we talked a little bit about immuno assays, particular with CDI that's been under development, which are promising certainly for future validations, but I think that at this point in time, that the animal bio assay is really the gold standard. But unfortunately, we're not supposed to discuss expense, but these analyses

are extremely expensive. They require particular expertise, and specific facilities, and these are not routine, so I think that also is going to factor into these deliberations, as well.

I think the issue of species and strain is an extremely important one that we've touched on today, and we've seen remarkable differences in the susceptibility of scrapie adapted isolates in the hamster system compared to CJD, so I think that that's an extremely important take-home lesson from what we've heard today. And I would underscore the fact that these studies need to be validated in the context of human infectivity. I think that's an essential component.

CHAIRMAN EDMISTON: So it's your take that these animal studies, by using these animal studies, we can validate transmission of TSE?

DR. TELLING: Yes, but in particular, it needs to be applied to the human infectious situation, CJD, sporadic CJD and ultimately, one -- we can't eliminate the possibility of variant CJD being a more global problem, as infectivity is detected in other

countries, and as Dr. Schonberger pointed out, we live in a global community, so it's something that we can't ignore.

CHAIRMAN EDMISTON: Well, I think we can address that specifically in the further questions, but the real issue is what's the feeling -- I want to get a sense for the panel in terms of their feelings relative to the animal studies for validation compliance. Yes, Dr. Gordon.

DR. GORDON: I think that since we're using the term "infectivity", that implies that we really need to have some in vivo results to help justify that. I don't think it can be done in a vacuum, and I think that all of us here have some serious concerns about the validity of the animal studies, or the animal model that we have in place right now, but it's the best that we have right now. And I think that as time goes on, and the models, hopefully, will become more valid, or pertain more closely to human infection, that'll be helpful. But for the time being, I think it needs to be a combination of in vitro and in vivo. And I think to

do it in a vacuum without the animal model, we could really miss significantly on it.

CHAIRMAN EDMISTON: Dr. Priola, what's your comment?

DR. PRIOLA: Well, I just want to second pretty much everything what Glen said and what Dr. Gordon just said; that the question is, is it reasonable for such an indication to be validated with animal studies? And I think it's eminently reasonable. It's the only approach we have in the TSE field currently that everyone agrees is sensitive enough, or as sensitive as we can get, to do this. There are in vitro things that are coming along, such as the CDI. There's an assay that's almost a PRC-like assay. I use that phrase loosely, but an assay that may have the potential to amplify undetectable levels of abnormal protein that may eventually be used as substitutes for the in vivo studies. But the in vivo studies would have to be done with human isolates, and I think that was again, as Glen said, and as I think others have said, again brought into a striking relief by the studies that were reported by Dr. Giles and

Dr. Prusiner.

DR. ARDUINO: I think right now, I mean, if we're looking for infectivity, we have to do these in vivo studies. I think what we have to come to agreement, though, are what the end-points are going to be.

CHAIRMAN EDMISTON: Do I get a sense then for question number one, the answer is yes? Would the FDA accept an answer that short, yes? All right. I think the --

DR. LIN: I think that sitting here listening to all the discussion, that the animal study is probably evident that that's the only model that's available. But now there's a question to us that asks Dr. Murphey, this morning in her presentation, she pointed out that each study has its own uniqueness. How we compare one study to the other study. I think if you can sort of elaborate that issue, that will be appreciated.

CHAIRMAN EDMISTON: Let's move on to question number two. "Discuss the relevancy of various design features of validation studies." And

this is where we start moving into the meat of it. Dr. Priola, would you like to comment?

DR. PRIOLA: Well, I was just nodding my head.

CHAIRMAN EDMISTON: You shouldn't do that.

DR. PRIOLA: Yes, I've got to stop nodding. Yes, in thinking about this, to me, the things that come immediately to mind is, these studies have to be -- if they're going to be dealing with instruments used in people, have to be based on human isolates of TSE; that this arbitrary time cutoff of 365 or 400 days, it has to go, and I know it's hard, and I know it's expensive, but it has to go as long as you possibly can in these animals, because that just increases the sensitivity of the assay. And I think one of the speakers had mentioned earlier this afternoon that when they validate for other infectious organisms, they always take the harshest, the toughest organism to test, and so you want to do that from the human point of view, as well. So something such as the isolate described again by Dr. Giles and Dr. Prusiner, is the kind of thing you would want to have

in those validation studies. So those, to me, are the three sort of starting points.

CHAIRMAN EDMISTON: Dr. Lurie.

DR. LURIE: Thank you. I think I would echo what I think Dr. Jarvis is saying, that in my mind, one of the reasons to talk about this is the more global issue of Mad Cow Disease. And I think as a member of the American community, it wouldn't make any sense to say something can get rid of TSE if it can't get rid of variant CJD. So it seems to me that whatever agents we're talking about should be able to decrease the infectivity of Mad Cow Disease, or it doesn't have any real context for me.

CHAIRMAN EDMISTON: Is anybody else nodding their head at me, that I can obviously see? How far down would you like us to break this? Would you like to break this down into the animal model, into the prion? All right. I have to really defer to my colleague who is the expert on this panel for this. In terms of the models that are currently available, what is your take, and also Dr. Telling's take, on the models that are currently available, that you feel

give us the best reproducibility from the perspective of a vendor who may have a proprietary device coming to market?

DR. TELLING: Well, I think it's clear in the transgenic approaches have revolutionized this field. Whereas, before people have used non-human primates as a means of detecting human infectivity, these sorts of animal models are obviously fraught with problems, and expense, and ethical concerns, not in the least, but I think it's clear that species variants and strains are an important consideration when assessing these very important questions. And we've been able to eliminate species variants completely in transgenic models by manipulating transgenic mice, creating transgenic mice. And wherever possible, these sorts of studies should be validated using the appropriate species and strain combinations.

CHAIRMAN EDMISTON: Now I understand, as scientists you have proprietary interest in your research and in the models you're using, but are you thoroughly convinced then the use of these transgenic

strains represent the best opportunity for reproducible data in this arena?

DR. TELLING: Well, I certainly do, yes.

DR. PRIOLA: I would say so, too, with the caveat that by reproducibility you're testing the same strain in the same model. But the minute you change the strain, the reproducibility is going to differ. For someone else - every pair of infectious agent and transgenic mouse goes together. That should be absolutely reproducible. That's always been the case in in vivo studies in TSE, but if you start switching, mixing strains and transgenic mice, then reproducibility will become an issue.

CHAIRMAN EDMISTON: In terms of these transgenic strains, is there a limited availability of those strains in terms of vendors being able to use those strains, or having access to those strains?

DR. TELLING: To my knowledge, these lines are available. I don't want to comment on proprietary issues, because I'm not involved in that.

CHAIRMAN EDMISTON: These are readily available, Dr. Priola?

DR. PRIOLA: There are several labs now who have derived them, and you can get them. I don't know how readily available they always are, but you can certainly find people who have them.

CHAIRMAN EDMISTON: And what is the gold standard human strain? Is there a gold standard human reference strain?

DR. TELLING: Strain of mice?

CHAIRMAN EDMISTON: No. Prion.

DR. TELLING: No, I mean -- I think we know -- we're beginning to understand the prevalence of strains in the human population. I think it's hard to say. I think it's impossible to say right now whether there exists 200 strains, or two, or half a dozen strains, but there's not one --

CHAIRMAN EDMISTON: Any homogenate from a patient would be an appropriate strain in this transgenic model. Is that true?

DR. TELLING: No, I think the strain that causes variant CJD is undeniably a different strain than what some people refer to as classic sporadic CJD. And then, of course, you have familial instances

of prion disease which are also transmissible, which again behave like different strains. The question is how many different strains of sporadic CJD are there out there. And one influence in this regard is the polymorphism of Codon 129, which has been mentioned in passing at least this morning, and also the confirmation of the actual infectious protein, PrP scrapie.

CHAIRMAN EDMISTON: So as per Dr. Jarvis' comment, the more strains that were tested in this model would be the desirable way to go.

DR. TELLING: Yes. But having said that, the WHO, for example, has set aside or characterized particular human prion isolates biochemically and by other means, and I think that those would be at least a good starting place for these sorts of validation studies. Whether they include variant CJD or not, I can't remember.

CHAIRMAN EDMISTON: How about the powering component of these studies, in terms of the number of animals that you would need as a per sample size? I know we've had some discussion on that. Do you have

any take on this from your own perspective?

DR. TELLING: I would leave that to the statisticians.

DR. PRIOLA: I'm not a statistician, but for the studies that we do, I like to have 12 to 14 animals, as were presented today. But in the case where you want to get to higher sensitivity, you don't have to have 12 animals in the low dilution group, so if you take a brain homogenate and dilute it ten to the minus one, or ten to the minus two, odds are all those animals are going to die. You don't need as many animals there as you do with the dilutions further out, so that's something that could certainly be scaled to favor the further out you dilute the brain homogenate, the more animals you have, the greater the potential sensitivity for detecting a single infectious unit.

CHAIRMAN EDMISTON: Dr. Jarvis.

DR. JARVIS: I guess a question for the group is, if I was a manufacturer coming to you with a product that I say can disinfect TSEs, do I need to provide data on classic CJD, genetically transmitted

CJD, and variant CJD, or just one of them?

DR. PRIOLA: Yes, that's a really good question. I think you'd have to present it at least on sporadic and variant, because variant is the one that's transmissible through blood. Genetic TSEs may differ, because there's a group of genetic TSEs that's transmissible, and a group that's not readily transmissible, so when you say genetic TSE, my question is which of the 30 should we test? They're very rare. It's very hard to get hold of the material, so I would say most definitely classical CJD, and as Glen said, going to the WHO reference collection, which is there for just this sort of thing, is a good place to start for that, because it gives you -- I think they try to have a brain homogenate from several different types of classical sporadic CJD, as well as variant CJD. The genetic one I think is a tougher proposition.

DR. JARVIS: That's why I think the more narrow this term of whatever, reducing TSE infectivity may be reducing classic CJD, new variant CJD infectivity, then you're being very specific, and it

ultimately would be, I think, easiest for the manufacturers if there were a bank of strains that were available, that everybody could test. That way you don't have me testing mine, you testing your's, somebody else testing their's, and we don't know that they're even the same.

CHAIRMAN EDMISTON: That's not going to happen any time soon. Correct?

DR. PRIOLA: Well, there's two.

DR. TELLING: Yes, there's WHO reference. These reference materials are available, and they've been typed, biochemically typed. And like I said, I think that would be a good starting place.

CHAIRMAN EDMISTON: Okay. So animal model we decided is the relevant model. We've also discussed some of the design features, transgenic mice model. You discussed the importance of using a human, human prion strain, most likely WHO reference strain, which would be a good start. Are there any other components in terms of the design elements of the validation studies that members of this panel deem as important for consideration?

DR. JARVIS: I think the statistical power that you mentioned, and we probably need to get a statistician to take a closer look at that, because obviously if you're looking at 12 animals, one versus two is not a whole --

DR. PRIOLA: Especially if you look at 12 animals for two years, because by the end of that time you might have eight animals, just by intercurrent --

DR. EDMISTON: Well, let me just hone in on our colleague over there, Dr. Cohen. Could you go to the podium, please, and address the statistical component?

DR. SCHONBERGER: Our statistician, I understand, wasn't able to come and join us.

Dr. EDMISTON: But he agrees with everything that was said.

DR. COHEN: To some extent, the sample size depends on the goal of the study. So, for example, if you want to demonstrate a six log reduction that's going to imply a certain sample size. Seven or eight might require even a bigger sample size, because you need more dilution levels, you need

more animals overall. But as my colleague indicated, at the lower dilution levels, you may very well be able to get away with less, because they're all going to die fairly early on. So the magnitude of the effect that you want to see is going to have a huge driving force. One of the concerns I have, also, is how many strains are you going to do it? Are you all comfortable with doing exactly one strain throughout the whole study, one source of infection? I don't know. I think that's also going to add to the sample size, as well.

CHAIRMAN EDMISTON: Well, the panel has indicated that they would be most comfortable with a number of different strains being tested. And is that a consensus of this panel?

DR. JARVIS: Until studies are done to show that one strain is the biggest, worst, whatever.

DR. PRIOLA: Yes, with that qualifier. If you took the WHO reference strains and somebody did a test and found that Type 1 or Strain A was the worst, then I would be comfortable with testing the worst.

CHAIRMAN EDMISTON: Okay.

DR. COHEN: So I guess the point is the control curve might be tied to the strain, as well, so the sample size could go up very quickly.

DR. TELLING: The control curve would have to -- yes.

DR. COHEN: I mean, if you need a control curve for the strain because that's how you titrate the dilution level, then it could add to the sample size.

DR. TELLING: Absolutely.

CHAIRMAN EDMISTON: Okay. I think it may be difficult for us to put a number on this particular point, but I think the way we've looked at this in the past is that the power of -- the study should be powered to give us sufficient faith in a P value.

DR. COHEN: Well, is the same idea. What you want to do is maybe a confidence symbol of the log reduction. It's the same idea. You basically -- if the confidence symbol includes a number that's always been within six, that you're confident that you have a six log reduction, I think that's the idea. And that's equated to a P-value if you want it to be done

that way.

CHAIRMAN EDMISTON: Now when we talk about this log reduction, the six number comes up quite a bit.

DR. COHEN: I've seen it in European documents on virology, so that's one of the reasons I've seen it, but other people may have seen it in other context. I don't know.

CHAIRMAN EDMISTON: Is this a number the panel is comfortable with in terms of the data that's been presented today? Dr. Telling? No comment?

DR. PRIOLA: Do you mean a six log reduction being sufficient?

CHAIRMAN EDMISTON: Sufficient.

DR. PRIOLA: No.

DR. SCHONBERGER: Not if the brain is eight logs.

CHAIRMAN EDMISTON: Okay.

DR. GORDON: It would seem also that the six log reduction or whatever was only carried out to the defined period of time, so I want -- if you're going out for two years or longer if the animals live,

then maybe you need a greater reduction than that. And I think that was something that many people brought out over the day.

CHAIRMAN EDMISTON: If we consider a larger log reduction, then what time period do you feel is sufficient, because obviously, 365 days is not going to be a sufficient period of time based, in part, on some of the data that was presented here.

DR. COHEN: Does it depend on the animal model?

DR. TELLING: In these studies, I think what usually happens -- I mean, 365 is kind of arbitrary, and I think we know why, after a year they just said let's look what happened. But I mean, usually we go to the end of the life span of the animal, and we allow all the inoculated mice to either develop disease or die a natural death, a non-prion related death.

DR. MANGAIYARKARASI: Yes. In one of the studies I read about the mice, they did the study for about two years, and this is 365, but maybe we can extend up to two years, up to the life span of the --

CHAIRMAN EDMISTON: Which is pretty much the life span of mice, too.

DR. TELLING: I mean, 600 days for a mouse is pretty good. But then whenever you're looking at age of animals, and you're trying to determine neurologic dysfunction, then you run into problems. So we kind of -- because we know what we're doing. Right? So we reach a point where we strike a balance, and we sacrifice the animals, and at a certain point where we know beyond that point, any additional time is not going to help us.

DR. SCHONBERGER: There's a number of issues with regard to the log reduction, and that is, the log reduction of what step? I mean, I think the cleaning alone seems to have a significant effect, several logs, so when we talk about a company saying that their particular product has a major reduction effect, I don't think they have to go out into ten logs and so on. If they can show that their particular product can lower it four logs, or six logs, I think that would defend the statement that their product is effective in reducing the

concentration of --

DR. EDMISTON: I think that was the early comment, because these are all adjunctive procedures relative to the routine cleaning process. But I'm trying to get -- obviously, when proprietary devices or products come to the FDA, there has to be some effort for a claim. And that claim may be for a single prion element, CJD, or it may be a more comprehensive claim. Is it likely you'll see more comprehensive claims, or do you see more focused claims in the future?

DR. LIN: I don't know. That's up to the manufacturer to decide. Since I'm on the point, now maybe I want to ask the question here. I'm thinking that when you talk about agent step that you just mentioned, the agent step, are we talking about the neurosurgical instrument, or we are talking about suspect instrument that would need to have this step? That probably would need to have some distinction, too. You follow my question?

CHAIRMAN EDMISTON: Yes. And I think comes up in the second or third question. So at this

point, the panel is comfortable with the concept of using a transgenic mouse model, with World Health Organization reference strains, with a sufficient statistical power. Dr. Asher.

DR. ASHER: You know, I just want to clarify, the World Health Organization reference strains are not intended to be working reagents. That should be kept in mind. They're intended to be calibrants to compare one test with another, or one other reference material, or one working stock with another. If suddenly large volumes of reference material were requested from the World Health Organization, they'd run out very quickly. They only have about 1500 ampules of each material in a volume of about half an MLH, and at 10 percent suspension, so that you really can't count on the WHO collection for working materials. I just want to make that clear. They haven't been characterized for their heat stability, and they haven't been even fully titrated yet in a variety of transgenic mice, only in a couple of types of transgenic mice.

CHAIRMAN EDMISTON: This is sounding more

like 2002, Dr. Priola. Is there any other source?

DR. ASHER: The various research -- there are sources, and we have some hope of establishing U.S. references which would then be available in larger quantities, but the funding source is not yet secure.

CHAIRMAN EDMISTON: Do you think it would be difficult for a vendor to -- if we were looking at clinically relevant strains, would it be difficult for a vendor to secure sufficient material to conduct these tests?

DR. ASHER: Sporadic CJD should not be difficult to obtain. The problem would be in getting a sporadic CJD that has been characterized, and at the moment, I think one would have to rely on laboratories like Dr. Prusiner's, and Dr. Pierre Luigi Convetis at Case Western Reserve supported by the CDC. But in principle, getting uncharacterized sporadic CJD tissue should not be extremely difficult. There are laboratories that have already invested considerable effort into characterizing their own materials, so whether Dr. Prusiner's lab have enough

to spare from their own research, I couldn't answer. I suspect that would be more difficult to do.

CHAIRMAN EDMISTON: Would it be reasonable to suggest that these studies be conducted with clinically relevant strains, and the source must be documented when they submit the data to the FDA?

DR. ASHER: If you're going to use human material, of course you have to confirm the diagnosis, absolutely. But you'd have to have a control positive titration, anyhow, so that if it didn't transmit, you would know it.

CHAIRMAN EDMISTON: So I think that might be one way to deal with this issue of clinically relevant strain in which the source is documented to the FDA. Does that satisfy the FDA? It's a little better than 2002.

DR. JARVIS: I guess the question comes up, if a vendor did the test on a strain that they obtained independently and it wasn't from Dr. Prusiner or from another source, and they do the studies, and it looks like just an incredibly susceptible to their disinfection method, do you believe it?

CHAIRMAN EDMISTON: Well, that's the issue for the FDA. I mean, the point is that, are these strains readily available? For instance, from a collegial perspective, obviously you have a proprietary interest in some of these issues. If a competitor asked you for strains, would you feel an obligation to provide that competitor with reference strains? Dr. Prusiner, yes, sir? Let's get to the meat and potatoes of this, all right?

DR. PRUSINER: I have to think about this, but I have a different approach. I think when you -- I mean, the studies we showed you were not just on human strains. We used 263K, Sc237, we used animal strains in addition to human strains. We've done a lot of this work, so that it's not all confined. It seems to me that you need to broad -- and you guys are talking about having multiple strains. And you asked me how many strains of sporadic CJD have I looked at. And I said to you we've looked at one, we have a tremendous experience with this one brain. I think if you asked me to give out large quantities of that brain to other people so they could work with it, the

answer is no. The problem with the whole WHO concept is that it is finite, whatever human brain you use. Now we've thought about transgenic brains.

Transgenic brains with human PrP genes, so you're making human prions, that's fine for, I think, assays, standards for immuno assays, for instance. But I'm not sure that's fine for inactivation studies like what we're talking about here, because I kind of think that human proteins, human other junk in the homogenate, is better than mouse stuff in the homogenate. So my sense is that the manufacturers should get -- should start with a lot of brain material from the sporadic one case, two cases, whatever is practical, but they should carry out in parallel some animal work, for instance, I guess 263K or whatever, so that one can see that their product that you're talking about gives some reference across from one to another. So you have a strain that's in hamsters, it's being passaged continuously in hamsters. It has been worked on extensively. That might solve your problem.

CHAIRMAN EDMISTON: So you'd have one line

of trial looking at clinically relevant isolate from homogenate to a reference isolate, and comparing those two.

DR. PRUSINER: Right. Exactly. I think that's the minimum.

CHAIRMAN EDMISTON: I think that's reasonable.

DR. PRUSINER: I think that's how you get around this, because you've gone around and around about what you do with a non-renewable source. If it's micro bacteria, you can grow it and grow it. If it's polio virus, you can grow it, and grow it. Measles, you can grow it, and grow it. This is different with human brain material surrounding a human prion.

CHAIRMAN EDMISTON: So I think the issue that you brought up in terms of if they submit data that looks wonderful, there's also going to be this reference data with a hamster strain, which is an excellent point. This committee feel strongly about that one way or another?

DR. JARVIS: That's a good idea.

CHAIRMAN EDMISTON: That's a great idea, so I think that would be our recommendation. Is somebody writing this down? Did we miss any points on question two, so we can move on to question three?

DR. JARVIS: Just one other design feature. It would -- at least I would appreciate having as a part of the study, as a control where you did nothing, but something in-between where you just did some kind of disinfection step without sterilization, because I'd like to know if that has a ten to the sixth impact, and then your sterilization step has ten to the one after that.

CHAIRMAN EDMISTON: Does that seem reasonable to the panel? Okay. Can we move on to question number three? Dr. Lin, we're going to move on to question number three now.

"Of the three study end-points cited in the literature, log reduction in infectivity, mean incubation point, and survival curve, which, if any, of these end-points may be adequate for the validation of reducing TSE infectivity claim? How may clinical benefit be estimated from these end-points?" I think

we've been discussing this all around, but is there any further comment, or any comment from panel members on this, on this end-point issue?

DR. JARVIS: You still want to use log reduction of infectivity.

CHAIRMAN EDMISTON: Yes. That's the gold standard.

DR. ARDUINO: The others don't really tell us anything.

CHAIRMAN EDMISTON: My reading of the presentations today and the information that I received, and also the statistical presentation suggests that one could make some correlation between that log reduction and some of the epidemiologic data that was presented today, so I think the log reduction appears to be a gold standard. However, Dr. Telling, let me ask you; are you comfortable with that log reduction concept?

DR. TELLING: I mean, the incubation time assay when linked to an end-point titration I think is certainly adequate.

CHAIRMAN EDMISTON: Yes, that's how it

looks to me. Any other comments? And I think therein lies the clinical relevancy of these log reduction studies. Any other comments on question three?

Question four - "What additional issues should be considered by FDA when evaluating claims for devices other than simple stainless steel surgical instruments? How can devices constructed from or including materials other than stainless steel, devices with complex shapes, devices with hinges or mated surfaces, or devices with lumens be addressed"? Now I know this is an important question for those of us clinically, because most of our devices are not the stainless steel pins. They're much more complex. So let me ask my clinical colleagues their take on this. Dr. Haines.

DR. HAINES: Well, it seems necessary that complex devices be examined for the ability to reduce infectivity. While the simple wire model may be extremely good for reproducibly producing infection, that's not really the issue. You have to do that, but then we have to know if we can eliminate the ability to produce infection. It seemed to me that there

could be a standard panel of devices, something with a lumen, something with a mated surface, perhaps two or three materials that would all need to be tested.

CHAIRMAN EDMISTON: Dr. Lurie.

DR. LURIE: Thanks. I would second that. I'm sure that in this business, there's kind of a standard panel of porous and non-porous materials, ceramics and different plastics, polyurethanes, and silicones, and they would all have to be sterilized. I think being able to sterilize one type of material, or at least thinking of porous and non-porous, being able to sterilize stainless steel but not titanium wouldn't make much sense.

CHAIRMAN EDMISTON: Dr. Coffey.

DR. COFFEY: Yes. I mean, trying to look at this like an ordinary medical device, we've been talking mostly about efficacy, but safety also has to do with the damage that might be done to the device. So stainless steel, a simple stainless steel instrument might survive the treatment with any putative device quite well. But a titanium instrument, or an instrument with a particularly

critical finish, or an instrument that has fiber optics, or any level of complexity might introduce risk to the patient if that instrument was completely sterilized or disinfected of prions, but if it didn't work as designed. You know, you put something into the patient and you can't see through it, or can't -- so that's something to consider. And I don't mean to throw that in as a wild card, but especially when talking about non-stainless steel, or composite instruments.

CHAIRMAN EDMISTON: Well, the question was how can we address this? How can this address be addressed from the perspective of the vendor is going to be developing a proprietary product. How can this be incorporated into his testing battery?

DR. COFFEY: Yes. And that would be some sort of standard compatibility and stability kind of testing, so you'd have a device made of perhaps a non-functioning dummy device made of these putative materials, and that some manufacturer, or some test laboratory can say that yes, the surfaces meet the same specifications, or it's not 10 percent lighter

than when it went in so that you haven't lost substance and so forth. I mean, these are engineering issues.

CHAIRMAN EDMISTON: Let me ask one of the members of our audience, because someone discussed the use of devices other than the stainless steel. And I believe, Dr. Marchand, do you want to make comment to that?

DR. MARCHAND: Well, there is an alternative to look at these problems. And the total carbon assay is an assay, a radioactive assay that is very, very sensitive to the pentagram level in terms of detecting the presence of organic material. If you have a total carbon assay with zero on a surface, whatever the complexity of it, it means there's no way that you have a prion there. So you can do it, but not necessarily have to use prions to verify this aspect of it.

DR. LURIE: But if you have protein on the surface, as we've learned today that there's going to be, then you're going to have carbon there, and that doesn't mean there are prions there --